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# Molasses Grass Induces Direct and Indirect Defense Responses in Neighbouring Maize Plants

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## Abstract

Plants have evolved intricate defence strategies against herbivore attack which can include activation of defence in response to stress-related volatile organic compounds (VOCs) emitted by neighbouring plants. VOCs released by intact molasses grass (*Melinis minutiflora*), have been shown to repel stemborer, *Chilo partellus* (Swinhoe), from maize and enhance parasitism by *Cotesia sesamiae* (Cameron). In this study, we tested whether the molasses grass VOCs have a role in plant-plant communication by exposing different maize cultivars to molasses grass for a 3-week induction period and then observing insect responses to the exposed plants. In bioassays, *C. partellus* preferred non-exposed maize landrace plants for egg deposition to those exposed to molasses grass. Conversely, *C. sesamiae* parasitoid wasps preferred volatiles from molasses grass exposed maize landraces compared to volatiles from unexposed control plants. Interestingly, the molasses grass induced defence responses were not observed on hybrid maize varieties tested, suggesting that the effect was not simply due to absorption and re-emission of VOCs. Chemical and electrophysiological analyses revealed strong induction of bioactive compounds such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene from maize landraces exposed to molasses grass volatiles. Our results suggest that constitutively emitted molasses grass VOCs can induce direct and indirect defence responses in neighbouring maize landraces. Plants activating defences by VOC exposure alone could realize enhanced levels of resistance and fitness compared to those that launch defence responses upon herbivore attack. Opportunities for exploiting plant-plant signalling to develop ecologically sustainable crop protection strategies against devastating insect pests such as stemborer *C. partellus* are discussed.

**Keywords** *Chilo partellus* · Induced defence · Volatile organic compounds (VOCs) · Maize landraces · *Melinis minutiflora*

## Introduction

Plants have evolved intricate defence strategies to protect themselves, directly and indirectly, against attacking herbivores. Directly, plants under attack, produce volatile organic compounds (VOCs), proteins and metabolites that repel phytophagous insects (De Moraes et al. 2001; Kessler and Baldwin 2001); whereas, indirectly, they emit VOCs which attract natural enemies of the attacking herbivore such as predators and parasitoid wasps (Heil 2008; Tamiru et al. 2011). Apart from their role in indirect plant defence, herbivore- or wound-induced VOCs may also serve as vital external signals to elicit defence responses in neighbouring plants to warn them of impending attack (Mutymbai et al. 2016; Ton et al. 2006). Generally, VOC-mediated signalling between plants involves four main stages, the signal release and transport by the emitter plant and absorption and perception by the receiver plant (Baldwin et al. 2006). Once the signal has been

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recognized by receiver plant, it may respond with changes in signal transduction, transcriptome, proteome, and metabolome which may enhance levels of direct and indirect plant resistance against insect attack (Baldwin et al. 2006; Heil and Karban 2010; Ton et al. 2006). Several studies of plant-plant signalling suggested that exposure to VOCs can induce or prime plants to have faster defence responses upon subsequent herbivore attack (Heil and Silva Bueno 2007; Himanen et al. 2010). For example, lima bean plants exposed to VOCs from beetle-damaged conspecific shoots experienced reduced herbivore damage and increased the growth rate (Heil and Silva Bueno 2007). Exposure of maize seedlings to VOCs from *Spodoptera littoralis* infested plants led to stronger induction of defence-related gene expression upon subsequent elicitation conferring reduced caterpillar feeding and development as well as attraction of a parasitic *Cotesia marginiventris* wasps (Ton et al. 2006). Recent studies have shown that VOC-mediated communication between undamaged plants may also lead to changes in the volatile profile of neighbouring plants with subsequent effects on the behaviour of herbivorous insect pests and their natural enemies (Dahlin et al. 2015; Glinwood et al. 2011; Ninkovic et al. 2019).

Our previous studies of maize intercropped with molasses grass, *Melinis minutiflora* (P. Beauv.), showed a significant reduction in stemborer infestation and increase in larval parasitism by *Cotesia sesamiae* (Cameron) (Hymenoptera: Braconidae) (Khan et al. 1997, 2000). This has led to the development of a plant volatile-mediated crop protection strategy, known as a 'Push-Pull' or 'stimulo-deterrent diversion-ary' against cereal stemborers for smallholder farming system in Africa (Khan et al. 2010). The strategy uses knowledge of plant chemistry and insect behaviour to manipulate agro-ecosystems in a manner that is unfavourable to pests, whilst simultaneously promoting crop yield through reduced pest damage (Hassanali et al. 2008; Khan et al. 2010). The 'Push-Pull' system involves intercropping maize with repellent plants, such as molasses grass or desmodium, while planting attractive trap plants, such as Napier grass or *Brachiaria* grass, around the borders of the main crop (Cheruiyot et al. 2018; Khan et al. 2010). The intercrop 'pushes' away ovipositing stemborer moths by releasing VOCs that indicate low quality of food or presence of competitors; whereas, the trap plants attract the pest and provide a resource for laying their eggs. However, when the eggs hatch on the trap crop, the larvae are unable to survive or their development is constrained, thus reducing pest populations (Khan et al. 2000, 2010). The bioactive compounds responsible for repelling the pests and attracting the parasitoids are constitutively released from intact *M. minutiflora* and include (E)-ocimene, (E)-4, 8-dimethyl-1, 3, 7-nonyl-2-one (DMNT), (E)-caryophyllene, humulene and  $\alpha$ -terpinolene (Khan et al. 2000; Pickett et al. 2006). Most of these VOCs have also been shown to be produced by maize in response to insect

herbivory and/or egg deposition and are implicated in plant's defence against the herbivore (Dicke and van Loon 2000; Tamiru et al. 2011).

Although the functions of plant derived VOCs from the companion *M. minutiflora* plant have been demonstrated in ecological pest management (Khan et al. 2010), their role in plant-plant signalling has not yet been established. Here, we investigated plant-plant chemical communication between undamaged *M. minutiflora* and different maize cultivars and its subsequent effects on direct and indirect defence responses of the crop against the stemborer *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) pest. Specifically, we determined effects of constitutive *M. minutiflora* VOCs exposure on volatile emission profiles of maize cultivars from different origins, i.e. maize landraces and commercial hybrids. Moreover, we examined effects of induced VOCs from the maize cultivars exposed to *M. minutiflora* on oviposition behaviour of stemborer, *C. partellus* and its natural enemy, the parasitic *C. sesamiae* wasp and identified bioactive compounds mediating the observed behavioural responses. VOCs emitted by *M. minutiflora* exposed maize plants could enhance levels of resistance in the crop if they deter stemborer pest and/or attract herbivore's natural enemies. As far as we know, this is the first study to consider role of constitutive VOCs from undamaged *M. minutiflora* on volatile emission profiles of neighbouring maize cultivars from contrasting origin and determine subsequent effects of the induced VOCs at three trophic levels. Earlier studies on maize focused on effects of plant-plant communication from herbivore damaged plants and tritrophic interactions therein.

We hypothesise that VOCs from intact molasses grass can induce changes in volatile profiles of maize cultivars leading to subsequent effects on the next trophic level and plant–stemborer–parasitoid interactions. Plants activating direct and indirect defences by VOC exposure alone, without actual herbivore attack, could realize improved levels of fitness compared to plants that launch defence responses after herbivore attack. The stemborer *C. partellus* is one of the most injurious pests of maize, *Zea mays* L. (Poaceae), in sub-Saharan Africa, causing yield losses ranging between 20 and 80% depending on age of the crop at time of infestation and pest pressure (Kfir et al. 2002). It is furthermore predicted that the relative importance of *C. partellus* in the Lepidoptera pest complex of maize will increase in future due to the changing climate (Ntiri et al. 2016, 2019; Tamiru et al. 2007). Plant-plant signalling could be exploited to develop novel and ecologically sustainable crop protection strategies against the devastating stemborer pest and hence enhance smallholder maize production in the region.

## Methods and Materials

Study Site Studies were carried out at the Thomas Odhiambo Campus of the International Centre of Insect Physiology and



Ecology (icipe), Mbita point (0°25'S, 34°12'E, 1200 m a.s.l. and around 900 mm annual rainfall). The site is situated on the shores of Lake Victoria in western Kenya, where *C. partellus* is the key insect pest of maize (Khan et al. 2006).

**Insects and Plants** *Chilo partellus* were obtained from the insect mass rearing unit of icipe. The *C. partellus* colony was established from field-collected larvae and reared on a semi-synthetic diet as described by Ochieng et al. (1985). The mass-reared *C. partellus* culture was infused with a field-collected insect population every 3 months to avoid genetic decay and maintain the original behavioural characteristics of the species. Adults were sexed and gravid females selected for oviposition experiments and electrophysiology. Maize seeds of local varieties (landraces) 'Jowi-red' and 'Nyamula' were obtained from farmers in western Kenya; whereas, hybrid maize varieties 'WS505' and 'PH4' were obtained from commercial seed suppliers (Western Seed Company Ltd. and Kenya Seed Company Ltd., respectively). Seeds of molasses grass were obtained from push-pull field plots at icipe. Seeds of the maize varieties and molasses grass, *M. minutiflora*, were planted individually in pots filled with fertilized soil. The experimental plants were maintained inside insect proof screen houses under natural conditions (25 °C, 65% RH, 12 L:12D).

**Plant-Plant Communication** The plant-plant communication experiment was conducted by placing pots of newly planted maize varieties in between rows of 5–6 weeks old potted molasses grass until the exposed maize plants were 3 weeks old. The experimental plants were placed with a spacing of 75 cm between plants (within rows) and 100 cm between rows to ensure no direct plant to plant physical contact. After 3 weeks induction period, the plants exposed to molasses grass were removed and kept in a separate screen house until they were used in oviposition experiments and headspace volatile collection. Each maize variety was subjected to six different treatments, based on the time interval since removal from exposure to molasses grass VOCs prior to use in experiments, i.e. 0 h (immediately used), 24 h, 48 h, 72 h, 96 h and 1 week after removal. Ten replicates were done for each treatment.

**Volatile Collection** Volatile organic compounds (VOCs) from experimental plants, i.e. molasses grass, maize plants exposed to molasses grass and unexposed control plants, were collected using headspace sampling (Tamiru et al. 2011). The leaves of experimental plants were placed gently inside polyethyleneterephthalate (PET) bags (volume 3.2 l, ~12.5 mm thickness) through an open end of the bag. The PET bags were sterilized at 150 °C before use. Purified charcoal filtered air was pumped through the bottom of the bag at a rate of 600 ml/min. The adsorbent, Porapak Q (0.05 g, 60 / 80 mesh; Supelco) were placed at the outlet valve where air was

drawn at 400 ml/min. A low flow rate in the outlet valve allows adequate time and pressure for the Porapak Q to effectively adsorb the plant derived VOCs and minimize influx of air from outside through an open end of the bag. Prior to headspace sampling of *M. minutiflora* exposed maize varieties, the plants were removed from in-between rows of molasses grass after 3 weeks of growing together (induction period). Then, volatile samples were collected based on different lapse of time since removal from molasses grass intercrop, i.e. 0 h (immediately used), 24 h, 48 h, 72 h, 96 h and 1 week after removal. Control plants of corresponding maize varieties were planted in a different screen house under similar natural conditions but without exposure to the molasses grass. After 48 h of entrainment, volatile samples were eluted from Porapak Q with 0.5 ml of dichloromethane in 2 ml sample vials and kept in a freezer at -20 °C until they were used in bioassay and chemical analysis.

**Oviposition Bioassay** Two-choice tests were carried out using a modified methodology of Khan et al. (2007) in oviposition cages (90 × 60 × 60 cm) covered by a fine wire mesh netting. To determine *C. partellus* oviposition preference, molasses grass-exposed and non-exposed maize plants were placed inside the oviposition cages adjacent to each other and five gravid female *C. partellus* moths were introduced into each cage and allowed to oviposit overnight. The following morning, plants were removed from the cage and the number of eggs laid on each plant were counted under a light microscope at 6.5X magnification. A total of 10 replicates were done for each treatment.

**Four-Arm Olfactometer Bioassay** Responses of parasitic wasp, *C. sesamiae*, to VOCs collected from treatment and control plants were tested in a Perspex four-arm olfactometer (Tamiru et al. 2011). Aliquots of headspace samples (10 µl) from exposed and non-exposed maize plants were applied on to a piece of filter paper (4 × 25 mm) using micropipette and were placed at the two opposite ends of the olfactometer arms, while the remaining arms were solvent controls. Air was drawn through the four arms towards the centre of the olfactometer at a rate of 260 ml min<sup>-1</sup>. Mated female *C. sesamiae* parasitoids, without any previous exposure to plants or hosts, were transferred individually into the central chamber of the olfactometer using a custom-made piece of glass tubing. Time spent and number of entries into each arm was recorded using 'Olfa' software (F. Nazzi, Udine, Italy) for 12 min. The olfactometer was rotated every 3 min to avoid any bias due to directional effect. The experiments were replicated 12 times for each treatment sample.

**Gas Chromatography (GC) Analysis** Collected VOC samples were analysed using an Agilent 7890 GC instrument (Agilent Technologies) equipped with a cool on-column injector, a

non-polar HP-1 capillary column (50 m, 0.32 mm internal diameter, 0.52  $\mu\text{m}$  film thickness) and a flame ionization de-tector (FID). Aliquots (2  $\mu\text{l}$ ) of headspace samples were injected into the injector port of the GC instrument for analysis. The oven temperature was maintained at 30  $^{\circ}\text{C}$  for 2 min and then programmed at 5  $^{\circ}\text{C min}^{-1}$  to 250  $^{\circ}\text{C}$ . The carrier gas was hydrogen. The GC data were analysed using HP Chemstation software. The stereochemistry of linalool was determined by co-injections on GC fitted with a  $\beta$ -cyclodextrin chiral capillary column (Supelco, 30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness) (Tamiru et al. 2011). The GC oven was maintained at 40  $^{\circ}\text{C}$  for 1 min and then raised by 5  $^{\circ}\text{C min}^{-1}$  to 150  $^{\circ}\text{C}$ , where it was held for 30 min. After confirming successful separation of synthetic enantiomers, peak enhancement confirmed the presence of R-enantiomer in the headspace sample.

#### Coupled GC-Electroantennography (GC-EAG) Analysis

Coupled GC-EAG analysis was carried out using antennae of gravid female *C. partellus* and VOC samples collected from *M. minutiflora* exposed maize varieties and molasses grass. Five insects were used per selected representative headspace samples. The glass Ag-AgCl electrodes were filled with saline solution (composition as in Maddrell (1969) but without glucose). Female moths were chilled for 1 min before the antennae were excised. The tips of the antennae were removed to ensure a good contact when suspended between the two saline solution filled electrodes. Signals were passed through a high impedance amplifier (UN-06; Syntech, Hilversum, The Netherlands) and analysed using a customised software package (Syntech). The GC-EAG system in which the effluent from the GC column is simultaneously delivered to the antennal preparation and the GC detector was as described previously (Tamiru et al. 2011, 2015). Separation of the VOCs was achieved on a GC (Agilent Technologies, 6890 N) equipped with a cold on-column injector and a FID using a HP-1 column (50 m, 0.32 mm ID, 0.52  $\mu\text{m}$  film thickness). The oven temperature was maintained at 30  $^{\circ}\text{C}$  for 2 min and then programmed at 15  $^{\circ}\text{C min}^{-1}$  to 250  $^{\circ}\text{C}$ . The carrier gas was hydrogen. Outputs from the EAG amplifier and the FID were analysed using the Syntech software package. Peaks eluting from the GC column were judged to be bioactive only when they elicited consistent EAG activity in each of the five coupled runs (to avoid mistaking background noise for an electrophysiological response).

#### Coupled GC-Mass Spectrometry (GC-MS) Analysis

Aliquots of VOC samples were analysed on a capillary GC column (HP-1, 50 m, 0.32 mm i.d., 0.52  $\mu\text{m}$ ) directly coupled to a mass spectrometer (VG Autospec, Fisons Instruments, Manchester, UK) equipped with a cool on-column injector. Ionisation was performed by electron impact (70 eV, 250  $^{\circ}\text{C}$ ). The oven temperature was maintained at 30  $^{\circ}\text{C}$  for

5 min and then programmed at 5  $^{\circ}\text{C min}^{-1}$  to 250  $^{\circ}\text{C}$ . Tentative identification of compounds was made by comparison of spectra with mass spectral databases (NIST 2005) and confirmed through co-injection with authentic standards.

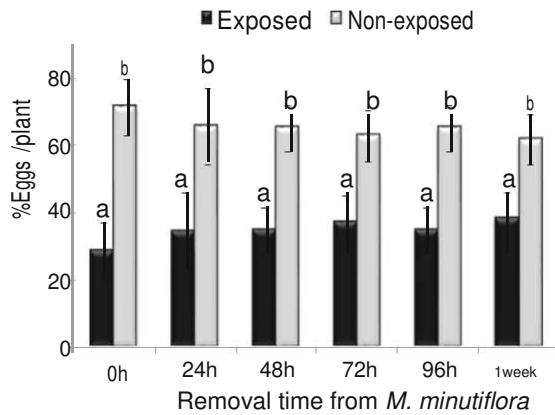
**Statistical Analysis** Two-sample (unpaired) Student's *t* test was used to compare significant differences in the number of *C. partellus* eggs between *M. minutiflora* exposed and non-exposed maize plants. Behavioural responses (attraction) of *C. sesamiae* to headspace volatiles from maize exposed to molasses grass and unexposed control were compared in a four-way olfactometer choice test. Bioassay data were generated using OLFA software, which provided a summary of time spent by *C. sesamiae* in each of the four olfactometer arms. The time spent (preference) data were then converted into proportions to address dependence of visiting time by *C. sesamiae* within the olfactometer fields, and then a log-ratio transformation ( $\log_{10}$ ) was applied to account for the compositional nature of the proportions (Aitchison 1986; Tamiru et al. 2011). The transformed data were checked for normality and then subjected to analysis of variance to determine significant differences between treatments and controls. The data analysis was implemented in R statistical software, version 3.2.3 (R Development Core Team 2015).

## Results

**Oviposition Preference (Two-Choice Test)** A significant reduction was observed ( $P < 0.05$ , Fig. 1) in proportions of *C. partellus* eggs laid on maize plants of the landraces 'Nyamula' and 'Jowi-red' that were previously exposed to *M. minutiflora* VOCs, compared to non-exposed plants, for all the treatment durations after removal from molasses grass exposure (0 h, 24 h, 48 h, 72 h, 96 h and 1 week after removal). In contrast, the proportions of eggs laid on *M. minutiflora* VOC-exposed and non-exposed hybrid maize varieties (WS505 and PH4) did not differ significantly ( $P > 0.05$ , Fig. 2).

**Behavioral Responses of Parasitoids to Maize Headspace Samples** In a four-arm olfactometer bioassay, gravid female *C. sesamiae* parasitoids were significantly attracted ( $P < 0.05$ ; Fig. 3) to headspace samples of plant volatiles collected from *M. minutiflora* VOC-exposed maize landraces, Nyamula and Jowi-red, compared to non-exposed and solvent controls, for all the treatments except for Jowi-red maize plants after 1 week of removal from exposure to *M. minutiflora*. In contrast, there was no significant difference in *C. sesamiae* preference to volatiles collected from the two hybrid varieties, WS505 and PH4, exposed to *M. minutiflora* VOCs and non-exposed ( $P > 0.05$ ; Fig. 4).

Nyamula



Jowi-red

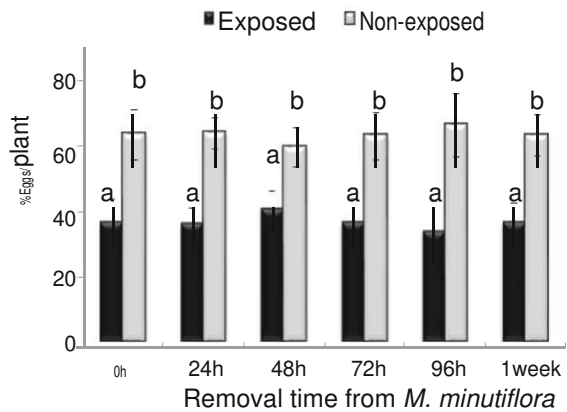


Fig. 1 Mean ( $\pm$  s.e.) percentage of *Chilo partellus* eggs laid on molasses grass-exposed and non-exposed maize landrace varieties, Nyamula and Jowi-red, in two-choice tests, at different removal time (N = 10). Means

with different letters above the bars are significantly different using Student's t test ( $P < 0.05$ )

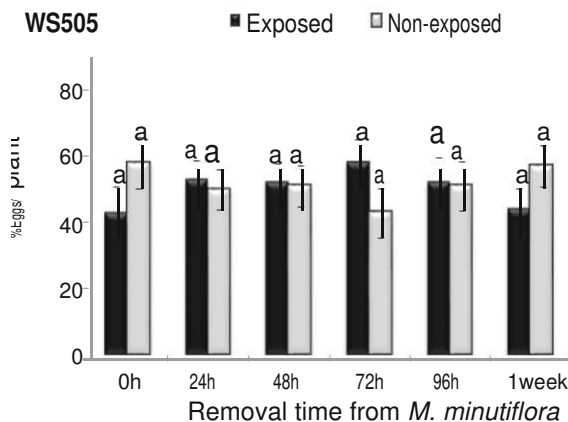
**Identification of Induced Volatile Compounds** GC analysis of the VOCs collected from *M. minutiflora* VOC-exposed and non-exposed maize landrace cultivars, 'Jowi-red' and 'Nyamula', revealed differences in volatile profile (Figs. 5 and 6). There was strong induction of bioactive volatile compounds such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) in *M. minutiflora* VOC-exposed maize landraces (Figs. 5 and 6). In contrast, volatile emission profiles of *M. minutiflora* VOC-exposed and non-exposed hybrid maize plants were very similar (Fig. 7). Coupled GC-EAG recording using the VOCs collected from *M. minutiflora* VOC-exposed maize landrace plants showed consistent responses from antennae of female *C. partellus* to induced VOCs such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). GC linked mass spectrometry analysis of volatiles from intact *M. minutiflora* revealed a variety of

bioactive VOCs associated with direct and indirect plant defences comprising monoterpenes (ocimene, nonanal), homoterpenes ((E)-4,8-dimethyl-1,3,7-nonatriene) and sesquiterpenes ( $\alpha$ -cedrene, (E)-caryophyllene,  $\alpha$ -bergamotene, (E)- $\beta$ -farnesene) (Fig. 8).

## Discussion

Our findings demonstrate that exposure of local maize landrace cultivars, 'Nyamula' and 'Jowi-red', to *M. minutiflora* VOCs elicits enhanced direct and indirect defence responses against *C. partellus* stemborers. As the experimental plants were in pots and there was no connection through the rhizosphere, VOCs rather than root exudates and microbiome are implicated in the plant-plant communication observed here. In oviposition bioassays, fewer *C. partellus* eggs were laid on *M. minutiflora* exposed maize plants compared to non-

WS505



PH4

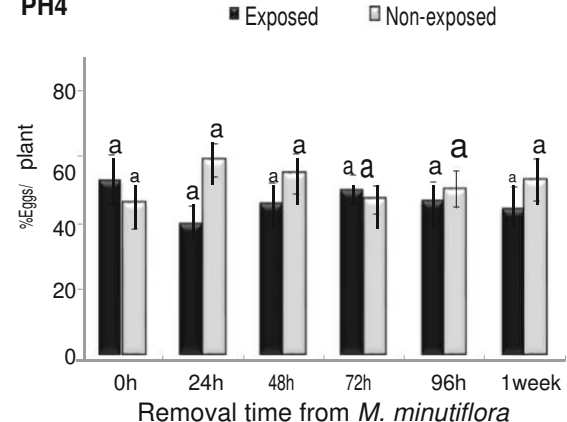


Fig. 2 Mean ( $\pm$  s.e.) percentage of *Chilo partellus* eggs laid on molasses grass-exposed and non-exposed hybrid maize varieties, WS505 and PH4, in two-choice tests, at different removal time (N = 10). Means with same letters above the bars are not significantly different using Student's t test ( $P < 0.05$ )

## Nyamula

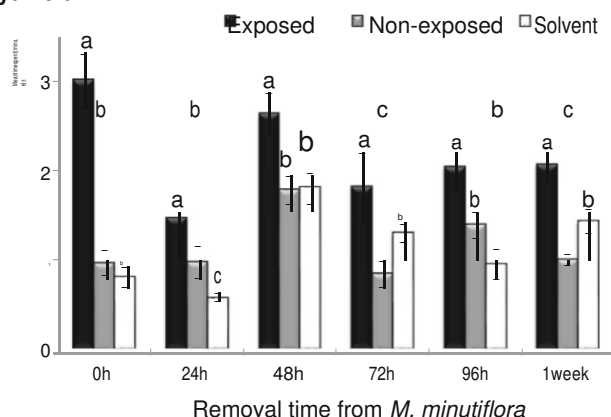
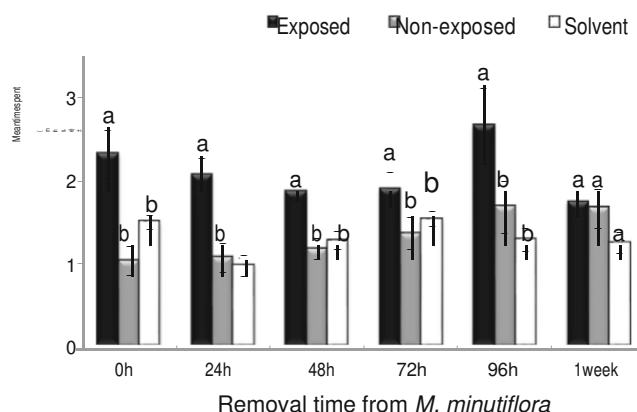


Fig. 3 Behavioural responses of female *Cotesia sesamiae* parasitoid in a four-arm olfactometer bioassay to volatiles collected from maize land-races 'Nyamula' and 'Jowi-red' exposed to *M. minutiflora* volatiles and unexposed control. Each parasitoid was observed for 12 min ( $N = 12$ ).

## Jowi-red



Time spent (min; mean  $\pm$  s.e.) by female *C. sesamiae* in the treatment and control regions of the olfactometer is shown. Error bars indicate standard error and different letters above bars show statistically significant differences based on the SNK test ( $P < 0.05$ )

exposed plants and the effect lasted at least for a week after exposure. The reduced oviposition preference for the exposed plants could be linked to the observed changes in volatile emission profiles of *M. minutiflora* exposed maize plants, however, changes in contact cues cannot be ruled out as moths were in contact with the plants in the oviposition bioassay. Plant preference by ovipositing moths is influenced by intrinsic properties of plants, particularly plant volatile chemistry, and central processing of olfactory signals by the insect (Renwick and Chew 1994; Nylin and Janz 1996; Konstantopoulou et al. 2002; Bruce et al. 2005). Volatiles collected from *M. minutiflora* exposed maize plants and analysed by GC-MS were different from VOCs of non-exposed plants. Furthermore, *C. sesamiae* parasitoid wasps

preferred headspace samples of volatiles collected from maize plants exposed to *M. minutiflora* VOCs than those from non-exposed control plants in olfactometer bioassays.

To compensate for their sessile nature, plants have evolved intricate mechanisms of detecting VOCs from neighbouring plants as one of the ways to sense their environment and respond accordingly (Ninkovic et al. 2019). Most previous research on plant-plant chemical communication has focused on VOCs mediated signalling by neighbouring herbivore- or pathogen-attacked plants (Ton et al. 2006; Baldwin et al. 2006; Karban et al. 2014). However, recent studies have demonstrated that exposure to volatiles from unattacked plants can also induce neighbouring plants to change their volatile emission profiles (Dahlin et al. 2015; Glinwood et al. 2011;

## WS505

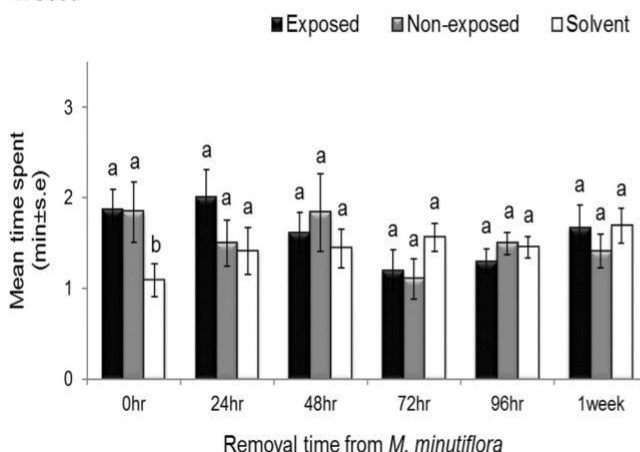
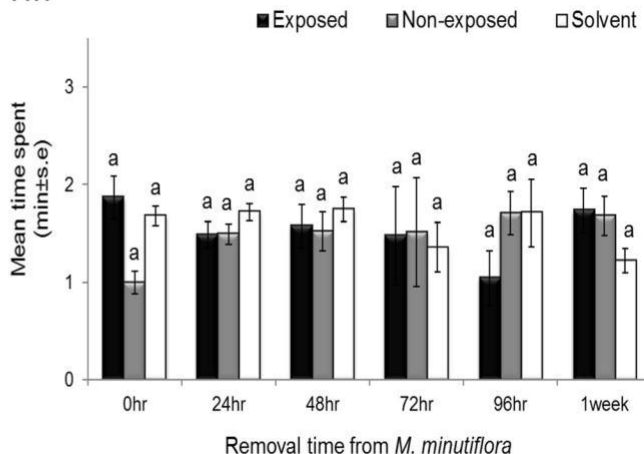


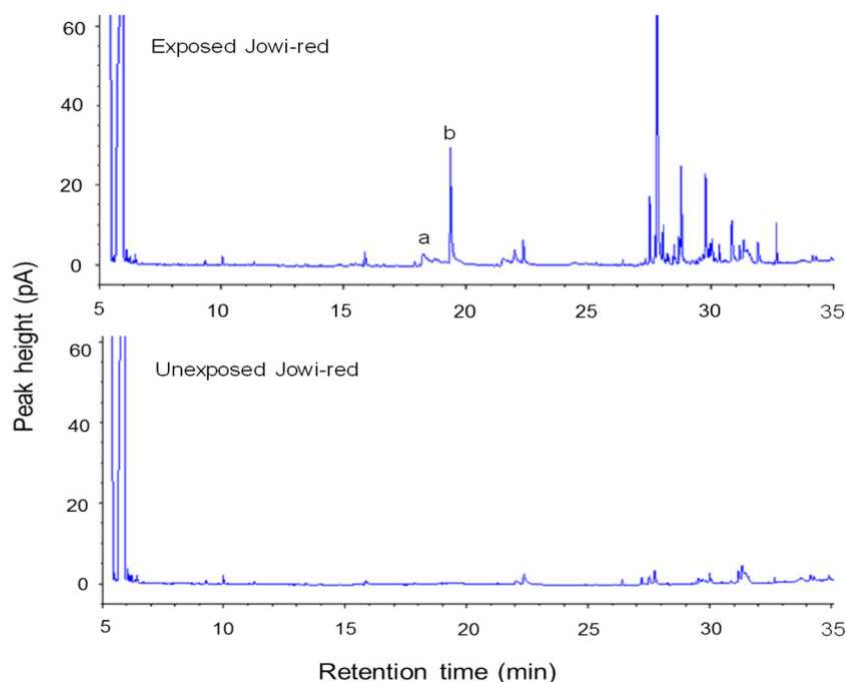
Fig. 4 Behavioural responses of female *Cotesia sesamiae* in a four-arm olfactometer bioassay to volatiles collected from maize hybrids 'WS505' and 'PH4' exposed to *M. minutiflora* volatiles and unexposed control. Each parasitoid was observed for 12 min ( $N = 12$ ). Time spent (min;

## PH4



mean  $\pm$  s.e.) by female *C. sesamiae* in the treatment and control regions of the olfactometer is shown. Error bars indicate standard error and different letters above bars show statistically significant differences based on the SNK test ( $P < 0.05$ )

Fig. 5 Representative GC profiles of volatile organic compounds (VOCs) collected from maize landrace 'Jowi-red' exposed to molasses grass and non-exposed controls (N = 6). Represented are the bioactive compounds: **a** (R)-linalool, **b** (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT)



Ninkovic et al. 2013). These neighbour-induced VOCs have been involved in insect-plant interactions such as repelling herbivores and attracting their natural enemies. For example, VOCs from onion plants induced neighbouring potato plants to emit greater quantities of two terpenoids, (3E,7E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) and nerolidol, which repelled green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Dahlin et al. 2015) and attracted 7-spot ladybird *Coccinella septempunctata*, an important natural

enemy of aphids (Vucetic et al. 2014). In related study, exposure of barley crop to volatiles from *Chenopodium album* L. (Amaranthaceae) and *Solanum nigrum* L. (Solanaceae) resulted in significant reduction in aphid acceptance rate when compared with unexposed plants in laboratory and field experiments (Ninkovic et al. 2009). Our current findings from the M. minutiflora -maize cropping system add to the limited number of studies where volatiles from an intact nonhost plant activate defence response in neighbouring host plants by

Fig. 6 Representative GC profiles of volatile organic compounds (VOCs) collected from maize landrace 'Nyamula' exposed to molasses grass and non-exposed controls (N = 6). Represented are the bioactive compounds: **a** (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT), **b** decanal, **c** (Z)-jasmone, **d** (E)- $\beta$ -farnesene, **e** (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT)

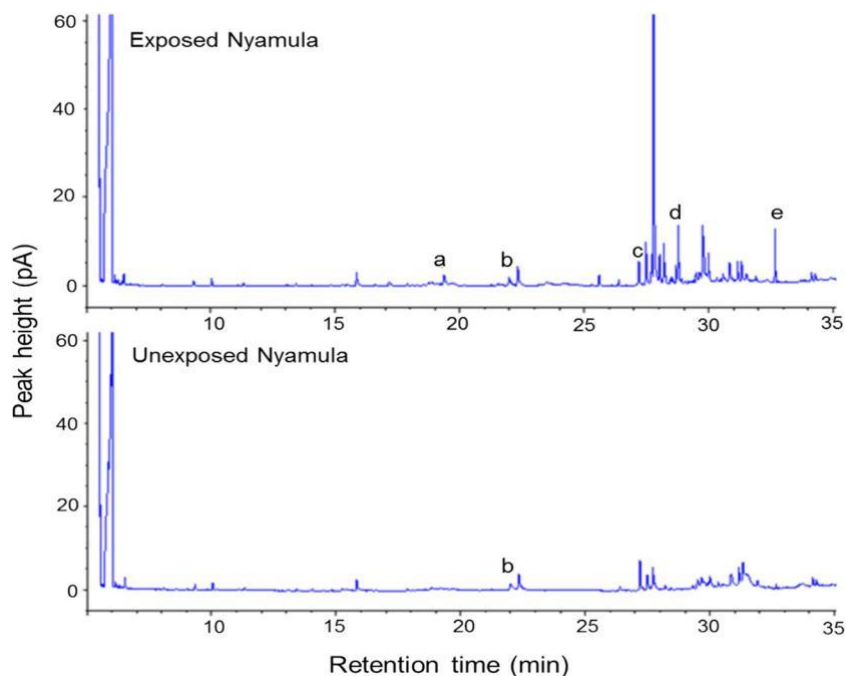
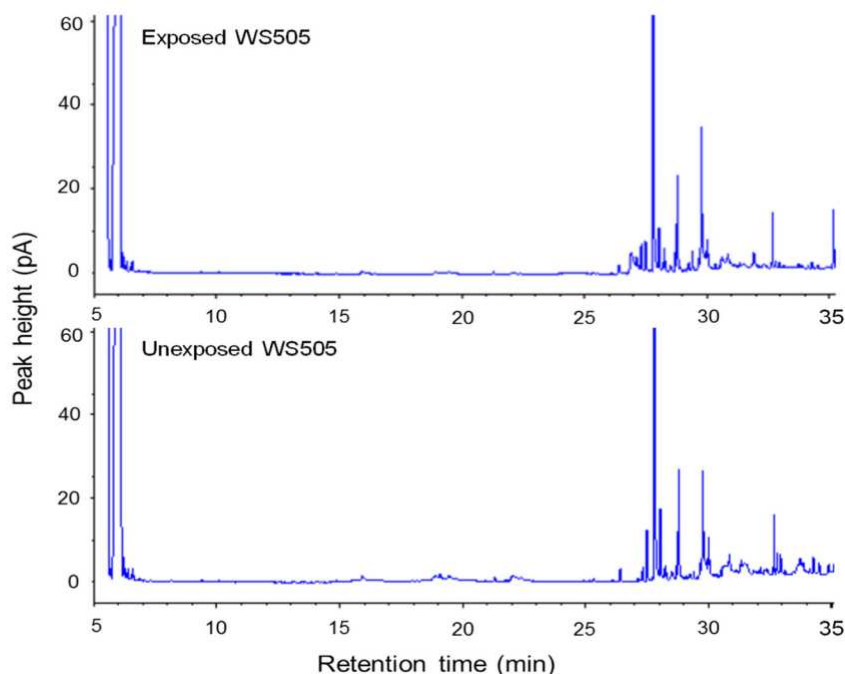




Fig. 7 Representative GC profiles of volatile organic compounds (VOCs) collected from the hybrid maize variety WS505 exposed to molasses grass and non-exposed controls (N = 6). Very little difference in volatile emission profile was found between *M. minutiflora* exposed and unexposed WS505 maize plants



changing their volatile profiles as well as influencing their interaction with organism on other trophic level (herbivore). It is likely that plant responses to VOCs have evolved because they were associated with neighbouring insect attacked plants, or even damaged parts of the same plant, but here the neighbouring plant releases them constitutively.

Our study also demonstrated that VOCs from intact *M. minutiflora* induce changes in volatile profiles that have a role in indirect defence responses of neighbouring maize land-race cultivars. In the olfactometer bioassay, female *C. sesamiae* wasps were significantly attracted to volatiles from maize landraces exposed *M. minutiflora* compared to

non-exposed control plants. This suggests the VOCs mediated communication between the plants could enhance the foraging efficiency of natural enemies and thus improve their ecological fitness on *M. minutiflora*-maize intercropped fields infested with host herbivore. Comparison of volatile profiles revealed strong induction of bioactive compounds such as (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT) in maize landraces exposed to *M. minutiflora* compared to non-exposed plants. Previous behavioural and electrophysiological studies established responses of *C. sesamiae* to these compounds (Bruce et al. 2010; Tamiru et al. 2011, 2012, 2015).

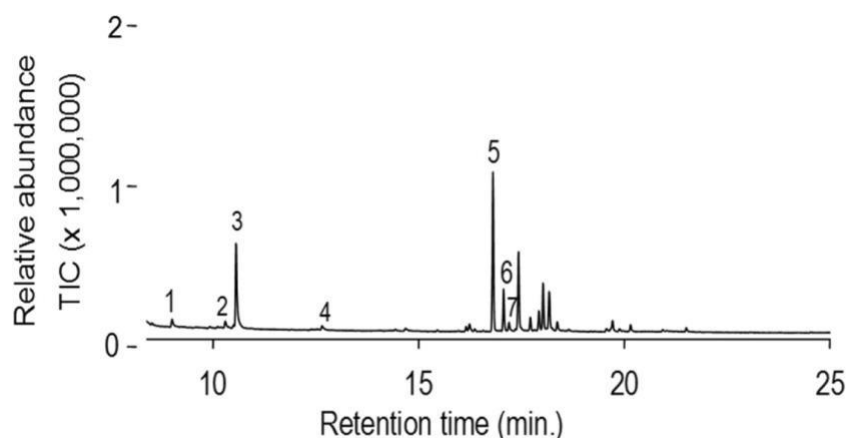


Fig. 8 GC-MS profiles of headspace volatiles from an intact molasses grass (*Melinis minutiflora* Beauv.). The identity of represented bioactive compounds is as follows: (1) ocimene (2) nonanal (3) (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) (4)  $\alpha$ -cedrene (5) (E)-caryophyllene (6)  $\alpha$ -

bergamotene (7) (E)- $\beta$ -farnesene. Our previous studies established these VOCs elicit behavioural and electrophysiological responses in the para-sitic *C. sesamiae* wasp (Tamiru et al. 2011, 2012, 2015)

*Melinis minutiflora* constitutively emits bioactive compounds including DMNT (Fig. 8) implicated as a cue for recruiting predators and parasitoids. Attractiveness of odours from *M. minutiflora* exposed maize landraces implies that *M. minutiflora* can trigger similar indirect defence responses in heterospecific neighbouring maize plants making them better prepared for upcoming insect attack.

The gas chromatography coupled electroantennographic (GC–EAG) recording confirmed that the VOCs induced after maize landrace exposure to *M. minutiflora* (e.g. (R)-linalool and DMNT) elicited consistent electrophysiological responses from antennae of female *C. partellus*. Our earlier studies have shown that the induced VOCs such as (R)-linalool, DMNT and TMTT are attractive to *C. sesamiae* in bioassays (Tamiru et al. 2011, 2012, 2015). This provides an explanation for the attraction of *C. sesamiae* wasps to *M. minutiflora* exposed maize plants and deterrence of *C. partellus* from egg deposition on the exposed plants. Insect responses to odour sources are mainly determined by the qualitative and quantitative composition of volatiles emitted by plants (Bruce and Pickett 2011; Tamiru et al. 2015). Neighbour-emitted VOCs could trigger changes in volatile profile of adjacent plants that are ecologically beneficial to the receiving plant when the induced production of volatiles deter herbivores and/or attract their natural enemies (Himanen et al. 2010). Our study demonstrates changes in maize volatile profiles, induced by neighbouring *M. minutiflora* VOCs, which inhibits oviposition by stemborer pest while attracting parasitic *C. sesamiae* wasps, a key natural enemy of the pest. Such VOC mediated defence activation among plants in proximity could have practical value in pest management especially under smallholder African agricultural systems as intercropping accounts for the majority of region's crop production practice.

Interestingly, the hybrid maize varieties used in this study (WS505 and PH4) appear to lack the ability to perceive and respond to molasses grass VOCs unlike the maize landraces. The absence of statistically significant difference in the number of *C. partellus* eggs laid between *M. minutiflora* exposed and non-exposed maize hybrids suggests lack of preference by ovipositing moths. Moreover, chemical analysis revealed very little difference in the volatile profiles of maize hybrids exposed to molasses grass and unexposed control, explaining the reason for the lack of behavioural response from the herbivore and its natural enemy to odours from hybrids exposed to *M. minutiflora* (Tamiru et al. 2011). Although the mechanisms by which plants accurately detect volatile cues from neighbouring plants are not well known, previous studies have indicated that plant-plant chemical interactions may occur in specific cultivar combinations of emitter and receiver within and between species (Ninkovic et al. 2019). For example, a study conducted to investigate effects of airborne interaction between different barley cultivars demonstrated that odour differences between barley cultivars could affect natural

enemy behaviour (Glinwood et al. 2009). Crucially, the difference in response between maize cultivars we observed demonstrates that the effect was not simply due to absorption and re-emission of VOCs because there would not be the marked differences between the hybrids and landraces as observed here. Moreover, some VOCs induced in maize landrace (e.g. TMTT in Nyamula) after exposure to *M. minutiflora* are not inherently emitted by molasses grass.

In summary, our findings provide evidence that VOCs inherently produced by adjacent *M. minutiflora* prepare the neighbouring maize landrace plants to better respond to insect attack by triggering their induced defences. Maize plants exposed to *M. minutiflora* VOCs were more attractive to *C. sesamiae* wasps and repellent to moths of the damaging herbivore, *C. partellus*. Recently a small but increasing number of studies have demonstrated volatile interaction between unattacked plants can elicit changes in volatile emission profiles of the receiving plants that reduce their attractiveness to insect herbivores and lead to attraction of herbivores' natural enemies (Dahlin et al. 2015; Glinwood et al. 2011; Ninkovic et al. 2019; Vucetic et al. 2014). Activating plant defences by VOC exposure alone, without actual herbivore attack, could enable plants to realize improved levels of fitness compared to plants that launch defence responses upon pest attack because defences are already deployed before arrival of the herbivore. This important ecological interaction can be exploited for better protection the crop from pest damage. For example, the 'push-pull' pest management strategy has been developed by exploiting inherent abilities of companion crops that produced VOCs that repel herbivorous insects from main crop while recruiting natural enemies of the pest (Khan et al. 2000). Induction of defence VOCs in the main maize crop itself, to deter pest oviposition while simultaneously attracting natural enemies through plant-plant communication could enhance their competitive ability in situations where there is a high risk of herbivory. These findings from the current study not only deepen our understanding on the chemical ecology of plant-plant interactions but also pave a way for development of novel and ecologically sustainable crop protection strategies against the devastating stemborer pests. Further studies are required to determine the mechanism of induction and whether the inducible defence trait is available in other maize genotypes including mainstream commercial hybrids. There is also scope for introgressing inducible defence traits into elite hybrid maize varieties which lack the trait, especially for resource constrained smallholder maize farmers in sub-Saharan Africa who cannot afford pesticides for crop protection. The prospects of exploiting VOCs' mediated plant signalling to improve crop resistance against insect pests has been demonstrated (Khan et al. 2010; Pickett and Khan 2016).

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